[From the *Journal of Anatomy and Physiology*, Vol. XI.]

ON THE EFFECTS OF SULPHATE OF ATROPIA ON THE NERVOUS SYSTEM OF FROGS.

By Sydney Ringer, M.D., *Professor of Therapeutics at University College*, and William Murrell, L.R.C.P., *Sharpey Physiological Scholar*.

In the course of an experimental investigation made with the view of elucidating the true nature of tetanus, we had occasion to repeat many of Dr T. R. Fraser's well-known experiments on the influence of atropia on the nervous system of frogs. We are induced to publish our observations; for though in the main they are confirmatory of those of previous observers, they differ from them in some respects.

In the first place we will speak of the tetanizing action of atropia. Dr Fraser has shown (1) that in frogs tetanic symptoms follow the subcutaneous injection of a dose of sulphate of atropia equivalent to about 1000th of the weight of the animal; (2) that this tetanus sometimes sets in on the second day, but more frequently on the third, fourth, or fifth; (3) that it varies in its duration from a few hours to seventeen days; (4) that it is due to the action of the drug on the cord (*medulla oblongata* and *medulla spinalis*).

The observations were made during the months of May, June and July. The frogs used for our experiments were with a few exceptions the ordinary *Rana temporaria*. We employed, except when the contrary is stated, a 1 in 20 solution of sulphate of atropia in water, the requisite dose being injected either under the skin of the back or into the axilla. The first twelve cases were observed thrice daily, between seven and eight in the morning, one and two in the afternoon, and five and six in the evening. In the subsequent experiments, observations were made much more frequently, with the view of determining how rapidly paralysis occurred, how soon it reached its height, and how quickly it declined. In some cases the animal was under almost continuous observation for many hours, in others the notes were taken every eight or ten minutes for the first hour, and hourly or every three hours subsequently. We may take this opportunity of explaining that whenever we employ the term

"pithed and pegged" we mean division of the cord by cutting, and destruction of the brain by the introduction of a piece of wood into the cranial cavity. We, like Dr Fraser, often obtained strong tetanus from the subcutaneous injection of atropia, but found that his dose (from $\frac{1}{735}$ to $\frac{1}{1250}$ of the weight of the frog) usually killed our animals instead of producing the desired result. With a smaller dose, however, namely, from $\frac{1}{1500}$ to $\frac{1}{2000}$, we were more successful. The tetanus in our experiments commenced earlier than in Dr Fraser's, our average period of onset being 20 hours; in one case it was well marked in 3 hours, whilst in the longest delayed it was 28 hours. With us, too, it lasted for a shorter time, for in one animal it continued only eight hours, and never in any instance exceeded five days. We imagine that these differences are due to the time of the year at which the observations were made. In the following table we give a summary of these experiments:—

TABLE I.

Why is the tetanus so long delayed after atropia poisoning? One writer, referring to Dr Fraser's paper, says that the paralysis of the motor nerves prevents the tetanic condition of the cord from displaying itself on the muscles, but Dr Fraser himself nowhere makes this assertion, and indeed his cases prove the contrary.

This proffered explanation we hold to be erroneous for the following reasons:—

I. Bezold and Bloebaum have shown that even with very large doses it is difficult to destroy completely the conducting power of the motor nerves, and it is obvious that as long as their conductivity is in the smallest degree retained, the tetanic condition of the cord must produce more or less tetanus of the muscles.

II. The onset of tetanus is delayed even in cases in which the dose of atropia is so small as to produce but slight paralysis, and it must be admitted that if the motor nerves are capable of conveying voluntary and normal reflex impressions they can conduct tetanic reflex stimuli. Thus in many of Fraser's cases there was never complete loss of reflex and voluntary power during the period which elapsed between the injection of the drug and the onset of tetanus. It will be found that of the cases in which he obtained tetanus there were twelve in which

the paralysis was incomplete; that in seven of the thirteen in which it had been complete, a partial recovery of reflex and voluntary power had taken place for a day or more before the onset of the tetanus. In eleven of our own twenty-six cases there was incomplete paralysis of reflex action and voluntary power, and in all these cases the onset of tetanus was delayed. In two cases, indeed, both voluntary and reflex power had been completely regained before the tetanus set in. In one case in which there had been complete paralysis, it began to improve five and a half hours before the commencement of tetanus.

III. If the poison be prevented from having access to certain limited regions by ligature of the nutrient vessels, the onset of tetanus is still delayed even in these protected parts. Fraser gives an account of four experiments, in which he adopted this mode of procedure. In three he tied the femoral vessels, and in one the abdominal aorta, before poisoning the animal, and yet tetanus did not occur in the protected limbs till twenty-two hours, fifty-one hours, three days, and twenty-two hours respectively.

We have tested the action of atropia on ten pithed and pegged frogs, in which, before poisoning, the abdominal aorta had been tied. The following was our mode of procedure:— We first divided the medulla by cutting through the occipito-atlantal membrane, and then passed a pointed wooden peg upwards through the foramen magnum into the skull, so as to destroy the brain. We then placed the animal on its back on the frog-board, and cut through the abdominal wall on one side, usually the left, taking care to avoid the abdominal vein. The intestines were then drawn aside, and the abdominal aorta having been slightly raised, was ligatured just above its bifurcation. The walls of the abdomen were brought together by sutures, and when the animal had recovered from the shock of the operation we injected our sulphate of atropia solution under the skin of the back. At the conclusion of our experiment we always ascertained by a careful post-mortem examination that the vessels had been securely ligatured. In every instance in which more than two or three drops of blood were lost, the operation was deemed unsatisfactory, and the animal rejected.

We give the results of these experiments in the following table :—

TABLE II.—*Observations on Pithed and Pegged Frogs with ligature of the abdominal aorta.*

Number of frog.	Weight in grammes.	Dose of sulphate of atropia in grains.	Proportion between dose and weight of animal.	Degree of tetanus.	Tetanus set in.	Tetanus lasted.	Loss of reflex action begun.	Loss of reflex action at its height.	Degree of loss of reflex action.	
27	32	$\frac{3}{20}$	$\frac{1}{3292}$	Strong	25 hours	52 hours	12 min.	27 min.	Nearly gone	Heart continued beating 3 days.
28	21	$\frac{3}{20}$	$\frac{1}{2160}$	Marked	9 hours	72 hours	12 min.	37 min.	,,	,, ,, ,, ,,
29	18	$\frac{3}{20}$	$\frac{1}{1851}$	None			20 min.		,,	,, ,, ,, ,,
30	20	$\frac{3}{20}$	$\frac{1}{2057}$	None			40 min.		,,	,, ,, ,, 2 ,,
31	18	$\frac{3}{20}$	$\frac{1}{1851}$	None			5 min.	48 min.	Quite lost	
32	20	$\frac{3}{20}$	$\frac{1}{2057}$	None			10 min.	42 min.	,,	
33	16	$\frac{3}{20}$	$\frac{1}{1646}$	None			5 min.		,,	
34	16	$\frac{3}{20}$	$\frac{1}{1646}$	None			5 min.		,,	
35	19	$\frac{3}{20}$	$\frac{1}{1954}$	Marked	$4\frac{1}{2}$ hours	24 hours	5 min.			Tetanus occurred as early and as marked in anterior as posterior parts of the body.
36	25	$\frac{3}{20}$	$\frac{1}{2572}$	None			7 min.			
						Average	12 minutes	38 minutes		

It will be seen from the table that in the three cases in which we obtained tetanus it was delayed for 25 hours, 9 hours, and $4\frac{1}{2}$ hours respectively.

In seven cases we tied the femoral vessels in the upper third of the thigh. Our mode of procedure was as follows:— We pithed and pegged the animal in the usual way, and having placed it in a prone position, carried an incision through the skin on the outer side of the thigh, when the vein coming into view was readily secured. By gently separating the muscles the artery was then seen and was tied as near the abdomen as possible, care being taken not to touch or otherwise injure the nerve. Finally the skin was brought together by a few sutures, and the operation was complete. In these cases the fact of the vessel having been securely ligatured was confirmed not only by the post-mortem examination, but usually by comparing by the aid of the microscope the condition of the circulation in the webs of the two feet. The details of these experiments are given in Table III.

It will be seen from the foregoing table that in the three cases in which we obtained tetanus, it occurred simultaneously in the two legs. In two cases it was equal in degree in the two legs, and in the third case it was stronger in the poisoned than in the unpoisoned limb.

We conclude then for these reasons that the late occurrence of tetanus in atropia poisoning is not due to paralysis of the motor nerves, but that it is owing to the cord being slowly affected. It appears that whilst the poison very quickly paralyses, it takes many hours, or even days, before it tetanizes.

In our experiments we obtained some rather unexpected results. Thus our observations lead us to conclude that atropia paralyses much more through its depressing action on the spinal cord than on the motor nerves. It is well known that considerable difference of opinion prevails on this point, some experimenters attributing the paralysis chiefly to the action of the poison on the motor nerves, and others to its action on the cord. Fraser in his article "On the connection between Chemical Constitution and Physiological Action," Part II, says, atropia "produces paralysis chiefly by affecting

the motor centres and sensory nerves," and our observation confirm this conclusion in respect of the motor centres (spina cord).

Thus in the experiments we have recorded in this paper after tying the abdominal aorta, or the femoral artery and vein, and then poisoning the animal, we found that paralysis set in as early, progressed as quickly, and became as complete in the ligatured as in the unligatured, and consequently poisoned limb.

It occurred to us that perhaps our experiments were made at a different time of year to those performed by other observers, and that this might account for the difference in our results. Our first observations were made in May, June and July, but with the view of solving the point we repeated many of them during the month of November. We tied the femoral artery and vein of the right leg of two frogs, and then poisoned them by injecting sulphate of atropia under the skin of the left axilla. To one we gave a dose too small to produce complete paralysis; to the other a much larger dose, namely half a grain, which caused complete paralysis in two minutes. In the frog to which the small dose had been administered both posterior limbs were almost completely paralysed, but the ligatured limb was a little the stronger after the poisoning. In the frog with the large dose, both hind limbs were absolutely paralysed in two minutes. We feel bound, therefore, to conclude that sulphate of atropia paralyses in great part by its action on the spinal cord.

Does atropia exert its action directly on the cord, or only indirectly through its influence on the heart and circulation? Is it a spinal depressant, or are the phenomena we have witnessed simply due to its action as a cardiac poison?

Atropia powerfully depresses the heart, slowing or even completely arresting its action. Even in cases in which the number of pulsations is reduced by only a half the heart does very little work, for on examination it is found that during diastole it becomes but slightly distended with blood, so that the circulation must be in reality almost at a standstill. That such is the case is also shown by a microscopic examination of the web of the foot, when the blood will be seen to be either

ationary, or to be moving very slowly in a few only of the rger vessels. It occurred to us, this effect on the heart and rculation might cause the paralysis of the spinal cord.

Vulpian found that ligature of the aorta just above the heart ıspended, in the course of a few hours, the excitability of the ord, and soon after impaired the conductivity of the motor nerves. he paralysis from atropia poisoning, however, comes on very uch more quickly than this, a circumstance which at first ght appeared at once to solve the question, and to show that ropia exerts a specific, or primary action on the cord. It ccurred to us, however, that it was not improbable that in ımmer when nutrient changes in frogs are performed much ore rapidly than in winter, the functional activity of the ord might be sooner affected by arrest of the circulation. Ve determined, therefore, to repeat Vulpian's experiment in modified form. We tested the condition of reflex action and oluntary movement in eight frogs, in which the circulation ad been arrested by mechanical means. In two of these ases the heart was cut or torn out from the chest, and in the emaining six the aortæ were securely ligatured just above neir origin from the bulb. The latter operation was per-ormed as follows:—The animal was pithed by cutting across ne medulla, and pushing a spigot of wood through the foramen nagnum into the cranial cavity. When the effects of the nock had completely passed off, we pinned the (brainless) nimal down on its back, and opened the thorax, by elevating ne lower end of the sternum and cutting through the adjacent oft tissues. The heart was then seen beating, and the peri-ardium having been opened, no difficulty was experienced in ipping a ligature under the aortæ close to the bulb, and ying them simultaneously. Finally, the sternum was replaced, nd the edges of the incision were brought together with a few utures. The operation was usually performed in a very few ninutes, and in most cases not a single drop of blood was lost. The experiment of arresting the circulation by removing the eart was even simpler. The brain having been destroyed as efore, the thorax was opened by one cut of the scissors and he heart was seized in the forceps, and at once removed. We nay mention that the success of the operation was confirmed

by a post-mortem examination, although such a step may hardly appear to have been necessary.

As the result of these experiments, we found that, on an average, the impairment of reflex action commenced in 13 minutes, and that the paralysis was complete in 37 minutes. It will be seen that our results differ considerably from those of Vulpian, a discrepancy which we then thought might possibly have been due to the season, and the condition of functional activity of the frogs, although our subsequent experiments have shown that such is not the case. As our experiments on circulation were made at the same time, and under identically the same conditions as our observations on atropia, they are obviously the best fitted for purposes of comparison.

By reference to the table (Table II.) giving the results of poisoning by atropia in frogs in which the lower limbs had been protected by ligature of the abdominal aorta, it will be seen that in four of these cases loss of reflex action was complete on an average in 38 minutes. These results accord in the most striking manner with those already described as resulting from the mechanical arrest of circulation, and they might be considered to afford a strong proof that the cardiac action of atropia is sufficient to account for the paralysis of the cord produced by this drug. Such, however, is not the case, for on examining the table it will be found that our experiments are in some respects unsatisfactory. The dose of atropia administered was small, so small, in fact, that in two cases the paralysis was never complete. It was therefore obviously necessary to ascertain whether larger doses would not produce complete paralysis in a shorter time. The experiments necessary for the elucidation of this point were made in November, and the opportunity was taken of instituting a series of comparative observations with the view of determining the effects of mechanical arrest of circulation in producing complete paralysis in brainless frogs. These results are given in the accompanying table:—

Pithed and Pegged Frogs poisoned with Atropia.

Date.	Weight of frog.	Amount in grains of sulphate of atropia.	Proportionate dose.	Paralysis complete.
Nov. 29	20 grammes	$\frac{1}{2}$	$\frac{1}{617}$	2 minutes
,,	24 ,,	$\frac{3}{10}$	$\frac{1}{617}$	4 minutes
,,	30 ,,	$\frac{3}{10}$	$\frac{1}{1543}$	9 minutes
,,	20 ,,	$\frac{1}{5}$	$\frac{1}{1543}$	3 minutes
			Average	4·5 minutes

Table showing the effect of mechanical arrest of circulation in pithed and pegged frogs.

Nov. 29				24 minutes
,,				55 minutes
,,				24 minutes
			Average	34 minutes

In these observations, sulphate of atropia caused, on an average, complete paralysis in 4·5 minutes, whilst mechanical arrest of the circulation required, on an average, 34 minutes. In the first of the atropia cases recorded in this table, we tied the femoral vessels before poisoning, and yet the paralysis became complete in both posterior limbs in 2 minutes.

We conclude, then, that atropia has a direct paralysing action on the cord, and does not affect it through its depressing action on the circulation.

In a paper in the volume of the *Medico-Chirurgical Transactions* for 1876, we have endeavoured to show that tetanus is not due to stimulation or an excited condition of the cord, but to a diminution or loss of resistive force in the reflex portion of the cord. This resistive force localizes the impressions conveyed through the nerves to the central nervous system, and when it is destroyed an impression can diffuse itself throughout the cord and produce a general evolution of force, which being conveyed by all the motor nerves to every muscle, produces tetanus.

We believe that the action of atropia confirms this view, or

at least is strongly opposed to the current notion that tetanus is due to an excited condition of the cord. Thus atropia, we believe, depresses the cord very powerfully. The resulting paralysis, which after moderate doses passes off in the course of a few hours, is followed by tetanus, sometimes in twenty-four hours, and at others much later. Now it appears to us almost inconceivable that a remedy should first paralyse the cord and then many hours later stimulate it. It may be urged that this delay in the appearance of the tetanus depends on the primary depression of the cord, and that the tetanus cannot occur till the paralysis has disappeared. This objection is obviously insufficient, for after a small dose of atropia the partial paralysis ceases in a few hours, or even in an hour, and the animal then seems quite well, but nevertheless the tetanus is delayed for twenty-four or more hours. Again, if this explanation is true, then the tetanus should never occur till the paralysis has disappeared, but this, though generally true, is not always the case, especially in frogs pithed and pegged before poisoning; for in these animals it often happens that paralysis, though far from complete, continues, and after some hours tetanus supervenes, at first very slight, so that a strong irritation excites tetanus, but a weaker irritation a coordinated reflex act. If tetanus depends on a stimulated condition of the cord, coordinated reflex acts should improve on the onset and with the increase in the amount of tetanus, but in reality the very reverse happens, for as tetanus grows stronger, coordinated reflex action simultaneously grows weaker and weaker.

Again, when unmutilated frogs made tetanic with atropia die, the tetanus continues to the last, growing weaker and weaker. This is still better seen in pithed and pegged frogs, for in them the tetanus also continues till all reflex action ceases. Now if tetanus depends on a stimulated condition of the spinal cord, it is obvious that as the cord gradually dies and consequently its functions become depressed, tetanus should cease, and give way to normal coordinated reflex action, but this does not happen; for on the contrary the tetanus grows weaker and weaker, still however, persisting until all reflex action becomes extinct. We have thus in atropia a drug that in large doses produces in pithed and pegged frogs progressive loss of power in the

cord, then after 24 or more hours tetanus sets in, which gradually increases in severity, coordinated reflex action simultaneously declining. We have, indeed, according to prevailing views a drug which paralyses the cord, and then after many hours the paralysis continuing or progressing it stimulates the cord.

When the paralysis is only slight, or when the animal completely recovers from it, then tetanic contractions of the muscles far exceed the amount of muscular action occurring in a natural coordinated reflex act; that is to say, during the tetanic paroxysm, there occurs a far greater discharge of nervous force in the cord than occurs in a normal coordinated reflex act, and this fact might be thought sufficient to justify the term stimulation of the cord. We have, however, already shown elsewhere that this view is probably incorrect.

When the paralysis is considerable and continues till the tetanus supervenes, the tetanic contractions are slight and the discharge of nervous force in the cord is probably less than occurs in a natural coordinated reflex act, and we have then paralysis with weak tetanus. The explanation of this combination is that atropia paralyses the reflex function as well as the resistive power of the cord. The paralysis of the reflex function of course weakens reflex action, whilst the paralysis of the resistive power allows a stimulus to spread throughout the reflex region of the cord, and hence every muscle becomes contracted and tetanus is produced; but as the reflex function is depressed the tetanus is weak.

In atropia we have a drug which quickly paralyses the reflex function of the cord, but requires a much longer time to diminish the resistive power of the cord; hence paralysis precedes and may even disappear some hours before the onset of tetanus.

TABLE I.—*Effects on Frogs of Subcutaneous Injection of Atropia.*

Number of frog.	Weight in grammes.	Dose of sulphate of atropia in grains.	Proportion between dose and weight of animal.	Degree of tetanus.	Tetanus lasted.	Tetanus first appeared.	Loss of reflex action.	
1	17	$\frac{3}{20}$	$\frac{1}{1748}$	Very strong	4 days	25 hours	Complete	Recovered. Tetanus increased as animal regained reflex power.
2	20	$\frac{1}{5}$	$\frac{1}{1543}$	None			Complete	Died.
3	21	$\frac{1}{5}$	$\frac{1}{1080}$	None			Complete	Died. Animals kept till rigor mortis and putrefaction set in.
4	26	$\frac{7}{20}$	$\frac{1}{1146}$	None			Complete	Died.
5	21	$\frac{7}{20}$	$\frac{1}{925}$	None			Complete	Died.
6	24	$\frac{1}{4}$	$\frac{1}{1481}$	Strong	5 days	22 hours	Complete	
7		$\frac{1}{4}$		Slight	3 days	11 hours	Complete	Recovered. Tetanus begun before return of voluntary or reflex power.
8		$\frac{1}{5}$		Slight		3 hours	Complete	Died. Never any return of voluntary or reflex power.
9		$\frac{3}{20}$		Marked		24 hours	Incomplete	Died. Up to time tetanus set in there was a fair amount of voluntary and reflex power.
10		$\frac{1}{4}$		,,	40 hours	28 hours	Incomplete	Recovered. Good reflex and voluntary power till tetanus set in.
11		$\frac{1}{5}$		,,	29 hours	23 hours	Incomplete	Recovered. Good reflex and voluntary power till tetanus set in.
12		$\frac{3}{20}$		None			Complete	
13	20	$\frac{1}{20}$	$\frac{1}{6172}$	None			Incomplete	Recovered. Paralysis slowly increased for 3 hours and then improved and almost recovered in 8 hours more.
14	19	$\frac{1}{20}$	$\frac{1}{5864}$	None			Incomplete	Recovered. Paralysis increased for 2 hours; almost recovered in 6 hours more.
15		$\frac{1}{10}$						Escaped. Paralysis begun in 6 minutes; at height in 15 to 30 minutes; begun to improve $2\frac{3}{4}$ hours.
16	19	$\frac{1}{10}$	$\frac{1}{2932}$	Slight	30 hours	24 hours	Incomplete	Recovered. Paralysis begun in 7 minutes; at height 15 minutes; begun to improve $2\frac{3}{4}$ hours.
17		$\frac{1}{10}$					Incomplete	Escaped. Paralysis begun in 8 minutes; at height in 15 minutes; begun to improve 3 hours.
18	14	$\frac{1}{10}$	$\frac{1}{2160}$	Marked	30 hours	24 hours	Incomplete	Recovered. Paralysis begun in 8 minutes; at height 16 minutes; begun to improve $5\frac{3}{4}$ hours.
19	18	$\frac{1}{10}$	$\frac{1}{2777}$	Slight	11 hours	24 hours	Incomplete	Recovered. Paralysis begun in 10 minutes; at height 30 minutes; begun to improve $2\frac{3}{4}$ hours. Completely regained voluntary and reflex power before tetanus set in.
20	17	$\frac{1}{10}$	$\frac{1}{2623}$	Slight	24 hours	24 hours	Incomplete	Recovered. Paralysis begun 16 minutes; at height 27 minutes; begun to improve $2\frac{3}{4}$ hours. Regained voluntary and reflex power before tetanus set in.
21	18	$\frac{3}{20}$	$\frac{1}{1851}$	Strong	8 hours	24 hours	Incomplete	Recovered. Paralysis begun 9 minutes; at height 30 minutes; begun to improve 2 hours. Almost recovered before tetanus set in.
22	15	$\frac{3}{20}$	$\frac{1}{1542}$	None			Incomplete	Recovered. Paralysis begun in 8 minutes; at height 34 minutes; begun to improve $1\frac{1}{4}$ hour.
23	12	$\frac{3}{20}$	$\frac{1}{1251}$	Very strong	67 hours	11 hours	Complete	Recovered. Paralysis begun 8 minutes; at height 20 minutes; begun to improve $5\frac{1}{2}$ hours.
24	16	$\frac{3}{20}$	$\frac{1}{1646}$	Very strong	61 hours	11 hours	Incomplete	Recovered. Paralysis begun 8 minutes; at height 20 minutes; considerable improvement 11 hours.
25	20	$\frac{3}{20}$	$\frac{1}{2080}$	Slight	35 hours	24 hours	Incomplete	Recovered. Paralysis begun 7 minutes; at height 30 minutes; begun to improve 2 hours.
26	15	$\frac{3}{20}$	$\frac{1}{1542}$	Very strong	62 hours	11 hours	Incomplete	Died. Paralysis begun 6 minutes; at height 20 minutes; much improved 11 hours.